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Low Temperature Effect on the Testicular Cell-components of the Common Indian Toad, *Bufo melanostictus* Schneider

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(Plate I; Text-figure 1)

THE degree of warmth, humidity, light and other climatic features in combination with the internal rhythm are responsible for controlling the sexual pattern of animals. The environmental temperature plays a great role in the reproductive physiology of poikilothermous animals such as fishes, amphibians and reptiles, although this appears to be lacking in warm-blooded birds and mammals (Bullough, 1951, p. 29). The potentially continuous type of spermatogenesis of *Rana esculenta* could be suppressed by decreasing the temperature during summer time, and the reverse during the winter induced activity (Galgano, 1934, 1935). Similar action on spermatogenesis was also proved to be true when *Triturus cristatus cranifer* (Galgano & Falchatti, 1940; Mazzi & Galgano, 1949), *Triturus viridescens* (Ifft, 1942), *Geotriton fuscus, Triturus alpestris, Rana gracea* and *Rana latestei* (Cei, 1942 a, b, and 1944) were treated with high temperature during winter and summer months. In the South American wood frog, *Leptodactylus ocellatus typica,* Cei (1948) and Rengel (1950) observed a temperature tolerance limit both in summer and winter and spermatogenesis is reported to be impaired if the frogs are treated beyond that range in both the seasons. However, Rengel (1950) was unable to observe any such effect when *Leptodactylus ocellatus reticulatus* was treated at high tempearture (± 30°C). Similarly, Cei (1944) could not induce spermatogenesis in *Rana arvalis* when it was treated with high temperatures during winter. Witschi (1924) reported that spermatogenesis is independent of environmental temperature in *Rana temporaria.* This was experimentally supported by Cei (1942, 1944) by keeping the frogs at high temperatures during the winter. But van Oordt (1956a, b) treated *Rana temporaria* at 5°C for two months and observed the absence of spermatogenetic activities. In *Telmatobius schreiteri* (Cei, 1949) and *Hyla raddiana andina* (Caruso, 1949) spermatogenesis is continuous and is not affected by the considerable low temperature of the high altitudes of the Andes mountains.

It is, therefore, evident that spermatogensis is dependent on the temperature in most of the Salientia, but with some specific variations. The above reports are from the temperate zone with the exception of some South American tropical examples. Consequently, it appears useful to study the influence of low temperature especially on tropical toads like *Bufo melanostictus* where spermatogenesis is continuous (Mondal & Basu, 1960; G. Church, 1960) and is not affected by an average temperature fluctuation of 38°-15°C.

Materials and Methods

Mature male toads, *Bufo melanostictus,* were collected from the vicinity of Calcutta and were brought to the laboratory the next day. The body weight and snout-to-vent length of all experimental animals varied from 28-32 gms. and 70-75 mm. respectively. Secondary sexual characters were carefully observed before treatment. A group of ten toads was allowed to stay in a controlled temperature room (±10°-15°) and another batch of five was kept at outside normal room temperature for one month. This experiment was performed during the months of August-September 1960 (Group A) and April-May 1961 (Group B). At the time of autopsy, body weight, snout-to-vent length, testicular weight and secondary sex characters were noted. The right testis of all the individuals was fixed and sectioned at 6μ and stained with hematoxylin

TABLE 1. OBSERVATIONS TAKEN FROM THE CONTROL AND LOW TEMPERATURE TREATED TOADS FOR THIRTY DAYS DURING THE MONTHS OF AUGUST-SEPTEMBER (1960) AND APRIL-MAY (1961).

	Relative Testis Wt. (in mg.)	Testis Tubule Diameter (in μ)	Spermatogenetic Stages‡						Percentage of Tissue Components		
			0	I	II	III	IV	V	Interstitium	Testis Tubule	Misc.†
August-September (1960) Group A											
Control (5)*	70	52.5	47.0	26.0	40.5	75.5	4.0	5.2	10.8	87.5	1.7
Treated (10)* ±10°-15°C 30 days.	67	45.5	48.2	25.0	42.5	70.5	2.7	4.0	20.35	78.6	1.15
April-May (1961) Group B											
Control (5)*	134	57.0	35.0	23.0	40.5	80.2	2.0	3.2	17.5	81.2	1.3
(Treated) (10)* ±10°-15°C 30 days.	135	56.5	37.0	22.5	27.5	75.7	2.2	4.0	18.2	80.5	2.3

*Figures indicate the number of toads used. †Includes blood vessels, vasa efferentia and some empty spaces. ‡Stage O = Primary spermatogonia at resting phase. Stage I = Secondary spermatogonia less than ten cells in a cell nest. Stage II = Secondary spermatogonia more than ten cells in a cell nest. Stage III = Primary spermatocyte. Stage IV = Secondary spermatocytes. Stage V = Spermatids.

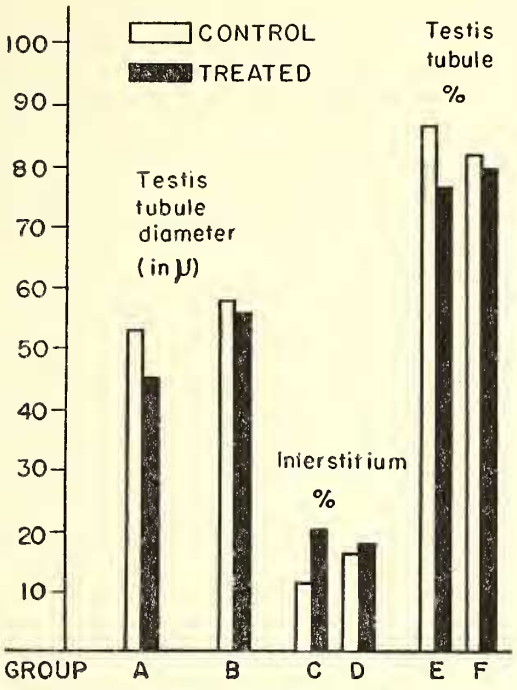
eosin. Different spermatogenetic stages were counted and the percentage of tissue components was calculated by a planimetric method. Tubule diameter and relative testicular weight were also recorded.

RESULTS AND DISCUSSION

The relative testicular weight of both control and treated toads of Group A (*vide* Table 1) is well within the normal range, as was previously reported by Mondal & Basu (1960) in dealing with the annual spermatogenetic cycle of the present species. The overall picture of the different spermatogenetic stages and the average number show no distinct difference caused by the treatment. A decrease, although not very remarkable, in the number of cell nests from stage III onward is observed among the treated toads of Group A (August-September, 1960). Sperms are embedded in the Sertoli cells and the vasa efferentia remain closed in all the treated and control toads during the months of August-September, 1960. The most prominent difference is noted in the testis tubule diameter, which is definitely narrow in the treated group (45.5μ) when compared with the controls (52.5μ). Similarly the percentage of interstitium has increased in the treated batch of animals (20.3%) as compared with that of the controls (10.8%). About 10% increase of the interstitial cellular components due to low temperature and an average shrinkage of 7μ of the tubule diameter are definitely significant (Text-fig. 1; Plate I, A & B).

In toads of Group B (April-May, 1961) both control and treated individuals show differences of insignificant nature in their relative testis weight, tubule diameter and also in their percentage of tissue components (Table I; Text-fig. 1). So far as the different spermatogenetic stages are concerned, an abrupt and sudden fall in the cell nest number of the secondary spermatogonia of stage II is noted in the treated group. The number of spermatocytes has also been decreased but not as significantly as have the spermatogonia. The sperms are found both in scattered and bundled condition in the tubular lumen, suggesting spermiating activity in the toads (Plate I, C & D).

The experiments of Galgano (1934, 1935) on *Rana esculenta* proved beyond doubt that temperature can induce or suppress spermatogenesis. On the other hand, in *Rana temporaria* Witschi (1924) and Cei (1942, 1944) believed that temperature caused no significant influence on spermatogenesis. Later, van Oordt (1956 a, b) and Galgano & Lanza (1951) proved that the influence of temperature exists in *Rana temporaria* if treated for a long period. All the above-mentioned observations are limited to the frogs of the temperate zone where normal environmental temperature is very low. On the contrary the frogs and toads inhabiting the tropical and subtropical zones generally show a continuous type of spermatogenesis and their temperature tolerance limit is also variable. This appears to be inherent and varies from species to species.



TEXT-FIG. 1. Illustrating the change in the tubule diameter (in μ), interstitium percentage and testis tubule percentage of control and treated toads in Groups A, C & E (August-September) and Groups B, D & F (April-May).

The normal cycle of *Rana tigrina* (Basu & Mondal, 1961) and *Bufo melanostictus* (Mondal & Basu, 1960; Church, 1960), inhabiting more or less the same climatic zone, show a good deal of difference particularly during the winter months. A similar condition is reported by Rengel (1950) with regard to a South American species of *Leptodactylus,* which shows continuous spermatogenesis. Rengel (1950) observed that if *Leptodactylus ocellatus reticulatus* is exposed to temperature higher than ±30°C there is no harmful effect on spermatogenesis, but this temperature appeared to be too high for the testicular cell divisions of *Leptodactylus ocellatus typica.* The present experiment on *Bufo melanostictus* reveals that very low temperature treatment in the warmest days (±36°-40°C) of the year (April-May, 1961) causes no significant effect except the decrease of the secondary spermatogonial number. But the toads of Group A (August-September, 1960) under similar treatment did not show any such remarkable fall in the cell nest number of stage II. On the contrary the tubule diameter and percentage of the interstitium was remarkably affected by low temperature in Group A. This proves that the role of low temperatures on male gonads of *Bufo melanostictus* is different during the summer and autumn months. In summer probably the spermatogenetic stages are more susceptible than is the interstitium of autumn. However, it is expected that prolonged treatment for several months may affect all the target organs during the summer and autumn months. In *Rana temporaria,* Witschi (1924) concluded that spermatogenesisis is to a large extent independent of the environmental factors and consequently depends on the internal rhythm determined by genetic factors. But experiments of van Oordt (1956) suggest that prolonged treatment may change the condition. Cei (1948) and Rengel (1950), from their experimental observations, are of the opinion that there remains a certain temperature tolerance limit in the spermatogenetic field of the Salientia. Moreover, from different reports so far available it appears that the temperature tolerance range of the Salientia varies from species to species even in the same climatic zone. The present experiment also suggests that in *Bufo melanostictus* seasonal variation causes some difference in the site of sensitivity and also that it has a wide range of temperature tolerance which is suitable for its adaptation to this tropical climate.

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SUMMARY

1. The common Indian toad, *Bufo melanostictus* Schneider, was treated with low temperature (±10°-15°C) during summer (April-May) and autumn (August-September) months of the year. The treatment was continued for one month.

2. No significant effect on spermatogenesis was observed except a sudden fall in the number of secondary spermatogonial cell nests of treated toads during the summer season.

3. The autumn toads after treatment showed a decrease in the diameter of the tubules and an increase in the percentage of interstitium in the testis tissue components.

4. Possible significance of the changes due to this treatment and some probable explanations have also been discussed.

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EXPLANATION OF THE PLATE

Plate I

(Photomicrographs of 6μ sections through the testis of *Bufo melanostictus*)

A. Section showing the hyperplasia of the interstitium (*vide* arrow) and narrow tubule diameter of treated toads during August-September (Group A). × 100.

B. Section showing the normal condition of the testis in the control toads of August-September (Group A). Note the undifferentiated interstitium and wide tubule diameter. × 100.

C. Testis sections of treated toads during April-May (Group B), showing the sudden fall of secondary spermatogonia (Stage II) in the follicles. × 100.

D. Section through the testis of control toads of Group B showing very little difference except the normal number of secondary spermatogonia (arrow indicates the stage II cell-nests). × 100.